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Differences in the Development of the Obese-hyperglycemic Syndrome in obob and NZO Mice*

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Summary. Marked differences were shown in the development of the obese-hyperglycemic syndrome in NZO and *obob* mice. — In NZO mice glucose tolerance decreases continuously with increasing age and body weight. — In *obob* mice three phases in the development of the obese-hyperglycemic syndrome are differentiated. In the first, dynamic phase glucose tolerance decreases and insulin secretion increases as does body weight. The intermediary or transitional phase, when the animals weigh about 55 g, is characterised by rapidly changing glucose patterns, i. e. an extremely poor glucose tolerance and extremely high serum insulin level is followed by improving glucose tolerance and decreasing insulin levels. In the third, static phase blood sugar values and serum insulin levels have nearly returned to those of the lean littermates. Body weight slowly decreases. The changes in glucose tolerance and serum insulin are parallelled by changes in islet cell morphology. The gluconeogenic capacity is increased during the dynamic and transitional phases, it declines during the static phase.

Différences dans le développement de l'obésité et de l'hyperglycémie chez les souris obob et NZO

Résumé. En étudiant le développement du syndrome obésité-hyperglycémie des souris *obob* et NZO, des différences prononcées ont été trouvées entre ces deux souches. — Chez la souris NZO, la tolérance au glucose décroît progressivement avec l'augmentation du poids et de l'âge. — Chez la souris *obob*, on distingue un développement du syndrome en 3 phases. Dans un premier temps dynamique, la tolérance au glucose diminue alors que la sécrétion d'insuline et le poids corporel augmentent. La phase intermédiaire ou transitoire — lorsque les animaux pèsent environ 55 g — est caractérisée par un changement rapide de l'allure des courbes de tolérance, c'est à dire qu'une mauvaise tolérance au glucose avec insulinémie élevée est suivie d'une amélioration de la tolérance au glucose et d'une diminution de l'insulinémie. Dans un troisième temps, statique, les taux de glucose sanguin et d'insuline circulante sont voisins de ceux des souris normales de même âge. Le poids corporel diminue lentement. Les modifications de la tolérance au glucose et l'insulinémie vont de pair avec des modifications morphologiques des cellules des îlots pancréatiques. La gluconéogénèse est accrue dans les phases dynamique et transitoire, mais diminuée dans la phase statique.

Unterschiedliche Entwicklung des Fettsucht-Hyperglykämie-Syndroms bei obob und NZO-Mäusen

Zusammenfassung. Ein Vergleich der altersbedingten Veränderungen der Glucosetoleranz bei *obob*- und *NZO*-Mäusen ergab folgende Unterschiede: Die Glucosetoleranz der NZO-Mäuse nimmt mit zunehmendem Alter und Körpergewicht progressiv ab. Bei *obob*-Mäusen lassen sich eine dynamische (I), eine intermediäre (II) und eine statische (III) Phase unterscheiden. Im Verlauf der Phase I nimmt die Glucosetoleranz ab, die Seruminsulinkonzentrationen und das Körpergewicht nehmen zu. Zu Beginn der Phase II (Körpergewicht ca. 55 g) ist die Glucosetoleranz sehr schlecht, die Seruminsulinkonzentrationen liegen sehr hoch; später verbessert sich die Glucosetoleranz etwas und die Insulinkonzentrationen sinken ab. Während der Phase III kehren Blutzucker und Seruminsulinkonzentrationen beinahe in den Bereich der bei Normaltieren des gleichen Wurfes gemessenen Werte zurück. Das Körpergewicht zeigt eine langsame Reduktion. Die Langerhans'schen Inseln zeigen während der Phasen I und II eine deutliche Hyperplasie, die sich im Verlaufe der Phase III weitgehend zurückbildet. Während der Phasen I und II sind die Aktivitäten gluconeogenetischer Enzyme deutlich erhöht.

Key-words: Spontaneous diabetes, mutation *obob*, *NZO* mice, diabetes in mice, obesity in mice, hereditary obesity, insulin, Beta-cells of pancreatic islets, adipose tissue.

American obese hyperglycemic mice (*obob* mice) and New Zealand obese mice (NZO mice) have been referred to as possible models for maturity-onset human diabetes. Both strains are characterised by hyperglycemia coexisting with hyperinsulinemia. However, previous investigations indicate that the syndrome in these two strains of mice cannot be regarded as identical. In *obob* mice the blood sugar during glucose load increases with increasing body weight and age of the animals [25]. In NZO mice, however, no correlation between glucose tolerance and body weight was reported [5]. Both strains of mice show hyperinsulinemia and increased pancreatic insulin content. However, the amount of insulin in the pancreas of *obob* mice [16] is about 6 times greater than in the pancreas of NZO mice [21]. Because of the differences in the development of obesity and hyperglycemia as well as hyperinsulinemia and pancreatic insulin content we studied carbohydrate metabolism *in vivo*, both in NZO and *obob* mice. In addition, the gluconeogenic capacity of the liver *in vitro* and the histology of pancreatic B-cells were investigated in *obob* mice.

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Materials and Methods

The following animals were used: American obese hyperglycemic mice (*obob* mice) and their lean littermates were obtained from The Jackson Memorial Laboratories, Bar Harbor, Maine, USA.

New Zealand obese mice (NZO) which originated from the strain described by Bielschowsky and Bielschowsky [1] were from our inbred colony. They were weaned at 21 days of age and separated according to their sex between 30 and 35 days of age. *Obob* mice were obtained at the age of 5—6 weeks.

For all studies only male *obob* and male NZO mice were used. The animals were housed in a room with a regulated temerature (25±2° C, 65% relative humidity) and artificial light (8 a.m. to 8 p.m.). 5 animals were kept together in any one plastic cage (floor area 23×17 cm) covered with saw dust. The animals were allowed free access to food and tap water. The food was in pellet form and produced by Intermast GmbH, Bockum-Hoevel, Germany. Food was composed as follows:

Composition:

20.00%	Concentrated protein, consisting of:
	50.0% Soja shred, extracted, toasted
	30.0% fishmeal
	10.0% powered cod
	10.0% fish solubles
30.00%	oats
24.00%	grain shredded i. e.
	10.0% wheat
	6.0% maize
	4.0% barley
	4.0% oats
5.00%	rusks ground
5.00%	tapioca ground
4.00%	wheat bran
3.00%	wheat germ
3.00%	clover green powdered
2.50%	distillers solubles
2.20%	whey powder
0.20%	vitamin concentrate
1.10%	minerals mixed
100.00%	

Weender Analysis:

crude protein	20.20%
crude fat	4.74%
crude fibre	3.50
ash	6.56%
H_2O	11.20%
N-free extracts	53.80%

Analytical carbohydrate content:
free carbohydrate 30.10%±5%

Addition of vitamin
(per kg diet are added)

Vitamin A	30000	I.E.	Ca-D-panthothenat	21	mg
D_3	6000	I.E.	Nicotinamide acid	60	mg
E	30	mg	Folic acid	2.2	mg
K_3	2.2	mg	Biotin	100	mg
B_1	9	mg	Cholinchloride	300	mg
B_2	9	mg			
B_6	9	mg			
B_{12}	30	mg			

Mineral content
(per kg diet are contained)

Calcium	14000 mg
Phosphorus	9200 mg
Magnesium	170 mg
Potassium	3900 mg
Sodium	1600 mg
(of this NaCl)	750 mg
Sulphur	110 mg
Ferrum	230 mg
Manganese	55 mg
Zinc	35 mg
Copper	11 mg
Cobalt	800 mg
Iodine	450 mg

Amino-acid content
(per kg diet are contained)

Glycerine	12600 mg
Alanine	12400 mg
Cystine	2300 mg
Serine	8900 mg
Methionine	3500 mg
Threonine	8900 mg
Valine	13200 mg
Leucine	17600 mg
Isoleucine	9000 mg
Asparaginic acid	21600 mg
Glutaminic acid	35700 mg
Arginine	16300 mg
Lysine	15200 mg
Phenylalanine	9900 mg
Tyrosine	8100 mg
Tryptophane	12800 mg
Histidine	7200 mg
Proline	13200 mg

Deviations in the composition of aminoacid patterns are ±5—20% according to raw materials.

Blood samples were obtained from the tip of the tail. For glucose determination, blood was collected in plastic centrifuge tubes without anticoagulant. Blood glucose was measured by the o-toluidine-method [12]. For serum insulin determination, blood samples of 2—3 mice were pooled, left for 2 h at room temperature, centrifuged and the serum stored at -18° C until assayed Serum insulin was measured according to Hales and Randle, method C [7]. Reagents were supplied by the Radiochemical Centre, Amersham, England. Crystalline bovine insulin (27 IU/mg, Farbwerke Hoechst, Germany) was used as a standard. On each sample, triplicate assays were carried out. Glucose tolerance tests were started at 8 a.m. and performed on non-fasted mice in the room with regulated temperature. 2.5 g glucose/kg body weight were given intraperitoneally as a 10% glucose solution. Blood specimens were taken at various intervals between 0 and 180 minutes after glucose load. $^{14}CO_2$-fixation was studied in liver slices according to L'age *et al.* (14). Na_2-$^{14}CO_3$ was supplied by The Radiochemical Centre, Amersham, England. Pyruvate carboxylase was assayed as described by Henning and Seubert [10]. Phosphoenolpyruvate carboxykinase was assayed according to Seubert and Huth [20]. Protein was determined as described by Lowry and coworkers [15]. For histological examination slices of pancreas were stained according to Schiebler and Schiessler [18] and investigated under UV- or visible light.

Results and Discussion

Body weight. In agreement with earlier observations by Crofford and Davis [5] we noted that during

the first months of age NZO mice gain body weight rapidly (Fig. 1a). Thereafter, weight increases more slowly but continuously by about 1.5 g per month. The highest body weight of approximately 70 g is reached at the end of the observation period when the animals are 12 months old.

weight, and a rapid decrease may be seen shortly before spontaneous death occurs.

Similar weight curves were observed by Westman [25]. The slightly different age-weight-relationship and maximal weight patterns may be a peculiarity of the Swedish colony of *obob* mice.

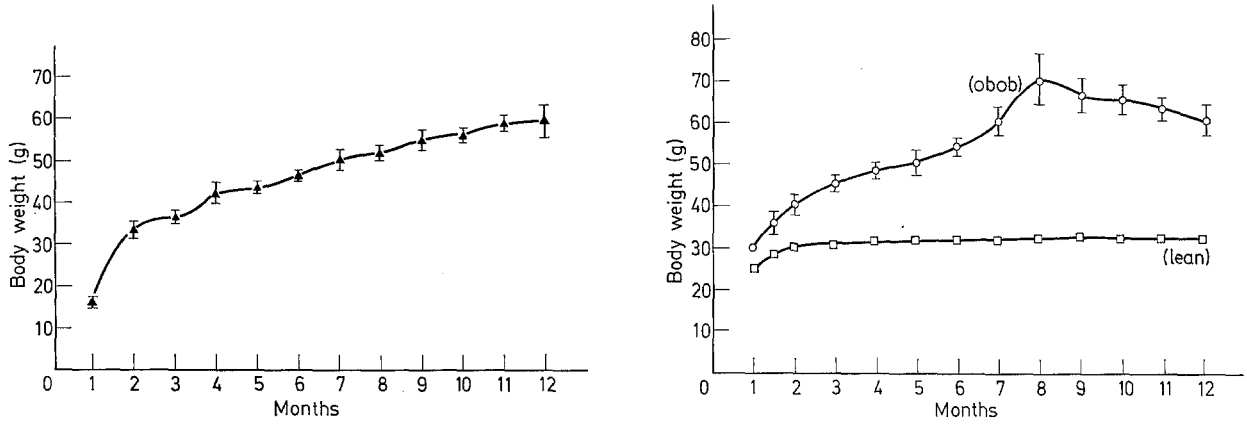

Fig. 1 a. Body weight of New Zealand obese mice (♂) of different ages. b. Body weights of American obese hyperglycemic mice (*obob*) and lean mice of different ages (♂). Mean ± SEM based on observations on between 20 and 50 animals

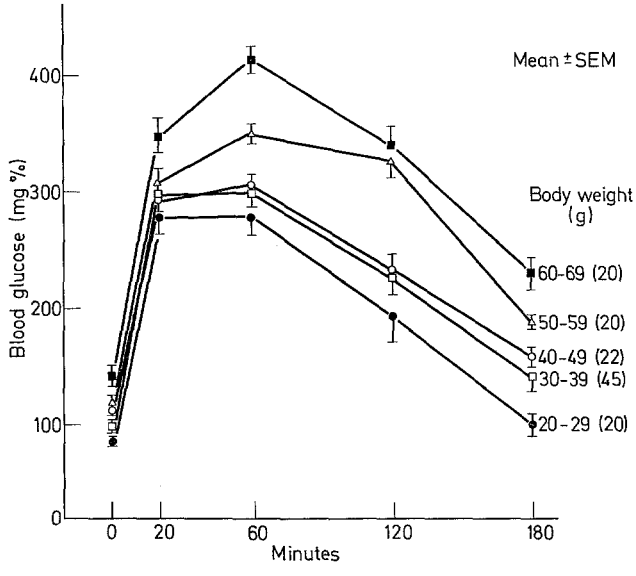

Fig. 2. Glucose tolerance test (2.5 g/kg body weight i.p.) in New Zealand obese mice with contribution to body weight. The number of animals is indicated in parentheses

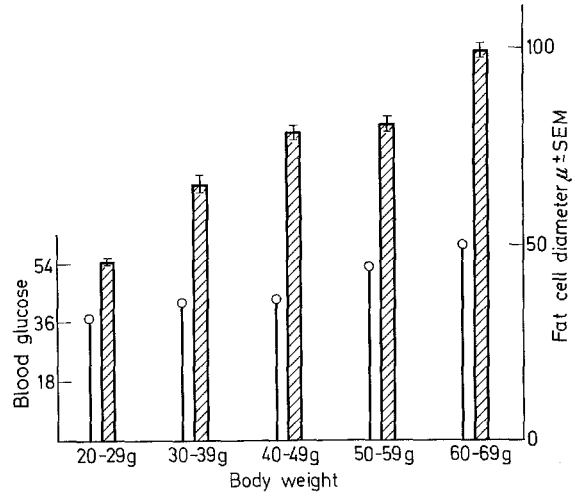

Fig. 3. Blood glucose and fat cell diameter with contribution to body weight in New Zealand obese mice. 0 = average blood glucose (mg% × 10^3) after glucose load between 0 and 180 min. Animals studied: blood glucose 20—45, fat cell diameter 10—18

While the lean littermates of *obob* mice do not change their average body weight of about 30 g after the second month of age, different periods in the development of obesity may clearly be distinguished in *obob* mice (Fig. 1b). A rapid gain in weight during the first three months of age is followed by a period of about 3—4 months in which weight increment is slower. Thereafter, the animals increase their weight again rapidly until the maximum is reached at about 10 months of age. Later there is a slight reduction in body

Blood sugar levels during glucose tolerance test in NZO mice. In NZO mice (Fig. 2) non-fasting blood sugar values slightly increase with growing body weight. In contrast to Crofford and Davis [5] we observed significant weight-dependent differences in blood sugar levels after glucose load, which were most remarkable in animals weighing more than 50 g. The slope after 60 min post glucose load is comparable in all groups studied, except in the group weighing 50—59 g which exhibits a more biphasic decline. At

the end of the test period the blood sugar in the group of animals weighing 20—29 g has returned to baseline, whereas in the other weight groups, the blood sugar values are significantly greater than at the beginning of the test. In this strain of mice there was not only a strong correlation between body weight and glucose tolerance but, as shown in Fig. 3, also between the average blood glucose levels after a glucose load between 0 and 180 min and the fat cell diameters. The same relationship was previously described in human obesity by Salans [17].

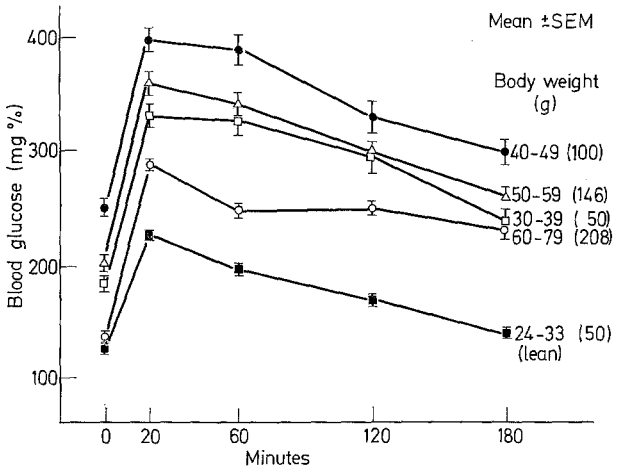

Fig. 4. Glucose tolerance test (2.5 g/kg body weight i.p.) in American obese-hyperglycemic mice (*obob*) with contribution to body weight

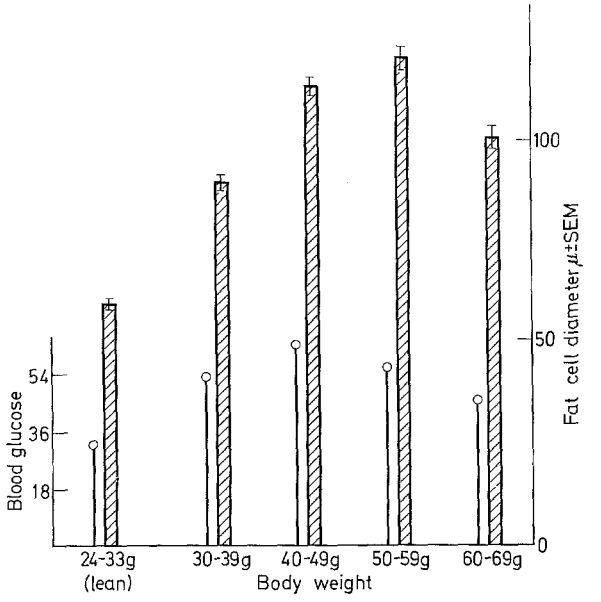

Fig. 5. Blood glucose and fat cell diameter with contribution to body weight in American obese-hyperglycemic mice (*obob*). 0 = average blood glucose (mg% × 10^3) after glucose load between 0 and 180 min. Animals studied blood glucose 50—208, fat cell diameter 7—52

Blood sugar and insulin levels during glucose tolerance tests in obob mice. In *obob* mice (Fig. 4) there is an increase in non-fasting blood sugar values only in the initial phase which parallels increasing body weight.

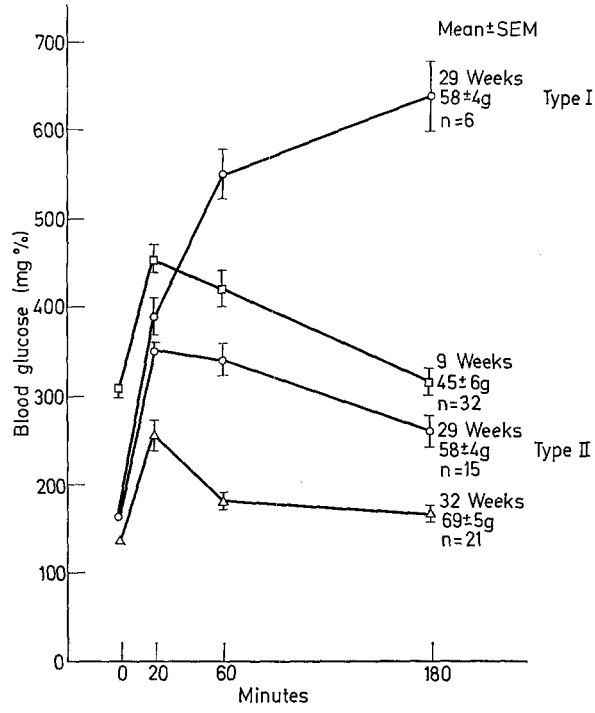

Fig. 6. Glucose tolerance test (2.5 g/kg body weight i.p.) in American obese-hyperglycemic mice (*obob*) with contribution to age (week) and body weight (g)

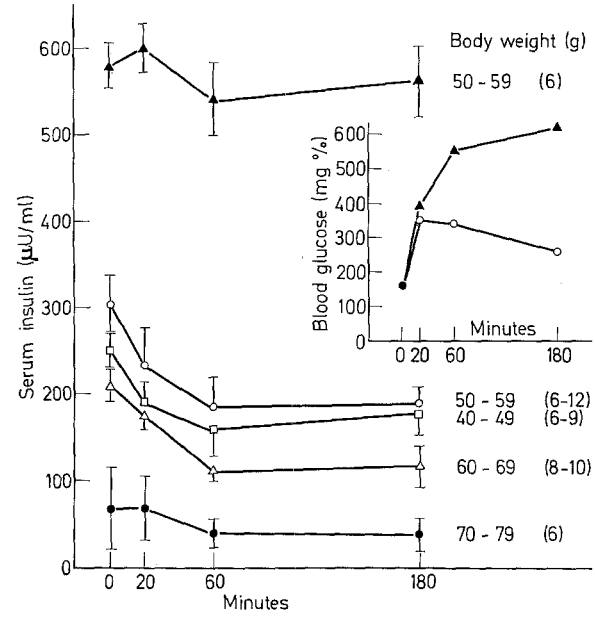

Fig. 7. Immunoreactive serum insulin (mean ± SEM) during glucose tolerance test (2.5 g/kg body weight i.p.) in American obese-hyperglycemic mice (*obob*) with contribution to body weight. The number of animals is indicated in parentheses

The peak of blood sugar values during glucose tolerance tests appears 20 min after glucose injection. The subsequent slope is similar in most groups, but is delyed in mice older than 7 months (60—79 g). In contrast to NZO mice *obob* mice of more than 50 g showed declining blood sugar values before and after glucose load. Therefore, in mice 6—7 weeks old (30—39 g) and 5—7 months (50—59 g) the reaction to glucose load may be equal in spite of the different age and body weight.

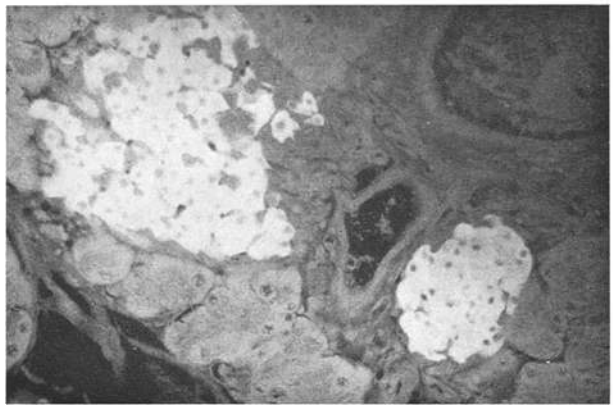

Fig. 8. Lean littermate. Normal sized islets with well granulated B-cells (white). Pseudoisocyanin stain, uv-light, × 500

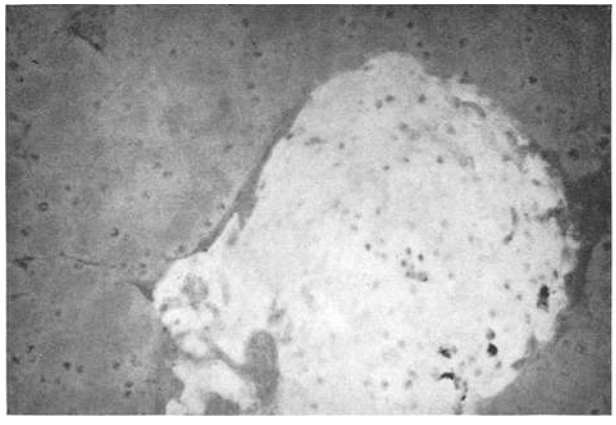

Fig. 9. *Obob*, 45 g. Moderately enlarged islet, well granulated B-cells. Pseudoisocyanin stain, uv-light, × 300

As shown in Fig. 4 the greatest blood sugar values are seen in 3 months old animals weighing between 40—49 g, the lowest blood sugar values being measured in animals of 8 months, weighing about 65 g. Consequently, it can be stated that, in contrast to NZO mice, glucose tolerance in *obob* mice is not diminished with increasing body weight. Furthermore, the correlation of blood glucose level to fat cell size observed in NZO mice was seen only in animals of less than 50 g (Fig. 5). In *obob* mice weighing 50—59 g, the average blood glucose level declined, whereas the fat cell size increased. Thereafter, the fat cell diameter as well as the average blood glucose declined with increasing body weight. This points to another difference between *obob* and NZO mice.

Although the great number of tests resulted in a small variance of the glucose values, a remarkable variability of blood sugar levels after glucose load was observed in the group of mice weighing 50—59 g. It could be shown that this variability is a consequence of two different patterns of glucose tolerance tests

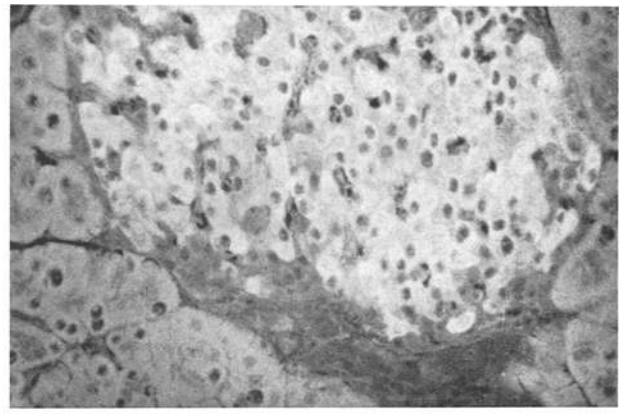

Fig. 10. *Obob*, 55 g. Moderately enlarged islets with partly degranulated B-cells (bright gray). Pseudoisocyanin stain, uv-light, × 500

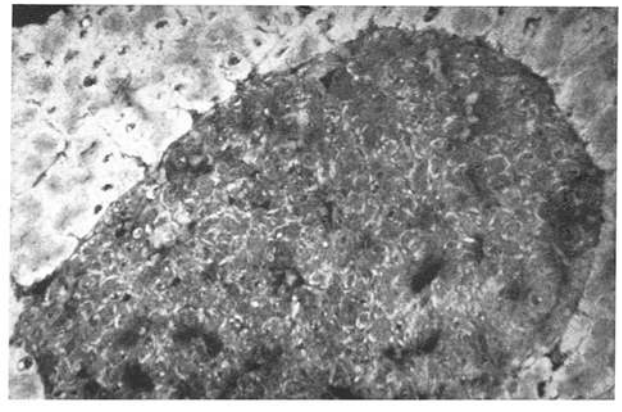

Fig. 11. *Obob*, 55 g. Enlarged islets with completely degranulated B-cells (dark gray). Cell membrane only fluorescent. Pseudoisocyanin stain, uv-light, × 300

which we could only distinguish in this weight group; one type is characterised by high blood glucose levels which rise continuously till the end of the test; the other type shows significantly lower levels with a peak at 20 min post glucose load and resembles the glucose curve in older animals. It could be shown in an additional study with a second group of animals (Fig. 6) that the first glucose tolerance test type precedes the second and that the two types are probably confined to only a short period of time, since the change in pattern occurred during an interval of 3 weeks. These

different types of glucose tolerance are parallelled by marked differences in serum insulin, as shall be discussed below.

As it is assumed that differences in glucose tolerance test are related to serum insulin levels, we measured serum insulin after glucose load (Fig. 7). In lean littermates the levels of insulin were not significantly different from zero-value at all time. In non-fasted *obob* mice serum insulin, like blood sugar, has a tendency to decline with age. However, the highest values are measured in animals weighing 50—59 g. It is surprising that the insulin levels showed no increase but a decrease after glucose load. It is possible that an early peak within the first 20 min of glucose load may have been missed. Again, we found not only the highest but the most variable insulin levels in the group of 50—59 g body weight. It could be shown that this variability was due to the presence of two clearly different groups, which corresponded to the animals with glucose tolerance tests of type one and type two, respectively. As demonstrated in Fig. 7, the animals with extremely high insulin levels have increasing blood sugar levels up to 180 min post glucose load. Moderately high insulin levels are seen in animals with relatively low blood sugar levels, which decline after the 20 min peak.

Histological examinations of the endocrine pancreatic tissue show that in lean littermates with low non-fasting blood sugar values, B-cells are filled with

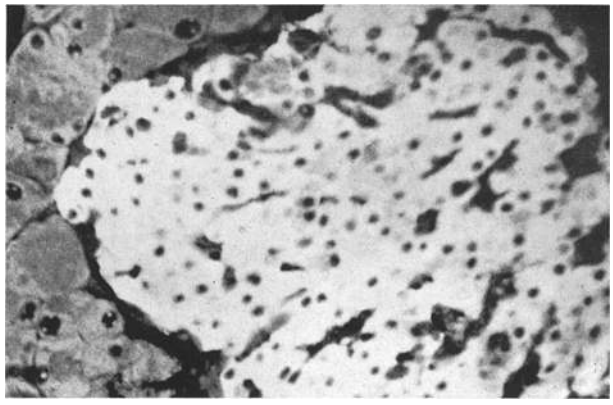

Fig. 12. *Obob*, 12 months of age. Enlarged islet, well granulated B-cells. Pseudoisocyanin stain, uv-light, × 500

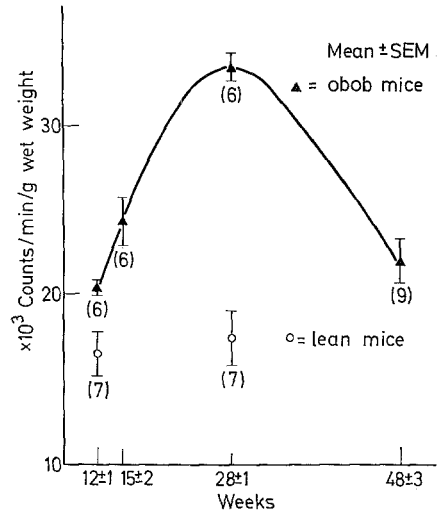

Fig. 13. $^{14}CO_2$-fixation in liver slices of American obese-hyperglycemic mice (*obob*). The number of experiments is given in parentheses

Table. *Activities of piruvate carboxylase and phosphoenolpyruvate carboxykinase in liver slices of lean and obob mice*

	Liver weight	Protein	Pyruvate carboxylase(a)		Phosphoenolpyruvate carboxykinase (b)	
	g	%	μunits/g wet weight	μunits/mg protein	μunits/g wet weight	μunits/mg protein
1.						
	1.56	16.7	1.35	8.4	6.67	39
	1.34	16.3	1.24	7.6	5.42	33
	1.02	16.0	1.49	9.3	4.82	30
	1.32	16.4	1.32	8.0	5.23	30
2.						
	3.24	16.9	1.26	7.4	5.70	34
	3.69	16.5	1.28	7.7	5.92	35
	3.41	17.0	1.45	8.5	5.59	33
	3.28	16.6	1.39	8.4	5.64	35

1. = lean
2. = *obob*
(a) = one unit is defined as the amount of enzyme catalyzing the carboxylation of 1 μmole pyruvate/min at 30° C in the assay described by Henning and Seubert [10].
(b) = one unit is defined as the amount of enzyme catalyzing the formation of 1 μmole phosphoenolpyruvate at 30° C in the assay described by Seubert and Huth [20].

Histological examinations of the endocrine pancreatic tissue show that in lean littermates with low non-fasting blood sugar values, B-cells are filled with

fluorescent material indicating a good granulation (Fig. 8). In *obob* mice weighing about 45 g and showing non-fasting blood sugar values of about 250 mg% (Fig. 9) the size of the islets is enlarged see also [2, 6, 9, 27]. The B-cells are well granulated, which is illustrated by the fluorescent spaces within the cells. In mice weighing approx. 55 g, two typical examples will be shown (Fig. 10). In one animal, the granulation is similar to that seen in the group of mice weighing 40—49 g (Fig. 11), yet in another animal only a small rim of fluorescent material is visible near the cell membrane. No blood sugar or serum insulin determinations were performed on the day the animals were killed, and we can only presume that the two different stages of granulation parallel the different levels of blood glucose and serum insulin. In mice of more than 10 months of age with increased glucose tolerance (Fig. 12), well granulated B-cells as well as degranulated B-cells can be seen.

Gluconeogenic capacity in liver slices. Insulin has been reported to be a suppressor of corticoid-induced increase in gluconeogenic enzyme activities [24]. Therefore, one may assume that the high blood sugar levels in *obob* mice are due to a defective muscular insulin responsiveness [22] rather than to elevated levels in gluconeogenic enzymes as a result of increased production of corticosterone [3, 8].

We therefore studied the gluconeogenic capacity of liver slices by means of CO_2-fixation. As shown in Fig. 13, CO_2-fixation is significantly increased in *obob* mice. In young *obob* mice, exhibiting the highest insulin levels, CO_2-fixation is smaller than in mice with with relatively lower insulin levels. This would confirm the hyphothesis mentioned above. However, the markedly decreased CO_2-fixation in old mice with the lowest insulin levels is very striking. Our results are in good agreement with those reported by other authors [4, 19, 26] who observed increased gluconeogenic enzyme activities in hyperglycemic mice. When measuring the activities of pyruvate carboxylase and phosphoenolpyruvate carboxykinase, the two enzymes which are involved in the abbreviated dicarboxylic acid shuttle, we did not see any difference between lean littermates and *obob* mice as shown in Table 1. These results are in contrast with those expected from our CO_2-fixation studies. The discrepancy may be due to a different intracellular distribution of pyruvate carboxylase [11] or to different levels of acetyl-CoA [13, 23] and needs further investigation.

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